FUNDAMENTAL STUDIES OF NEURAL STIMULATING ELECTRODES

First Quarterly Report
Covering Period August 29, 1994 to November 28, 1994
CONTRACT NO. N01-NS-4-2310

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This QPR is being sent to
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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1.0 INTRODUCTION AND SUMMARY

This report describes the work on NINDS Contract No. N01-NS-4-2310 during the period August 29, 1994 to November 28, 1994.

As part of the Neural Prosthesis Program, the broad objectives of the present fundamental studies are: 1) to evaluate the electrochemical processes that occur at the electrode-electrolyte interface during pulsing regiments characteristic of neural prosthetic applications; 2) to establish charge injection limits of stimulation electrode materials which avoid irreversible electrochemical reactions; 3) to develop an *in vitro* method, which can be applied *in vivo*, for determining the electrochemical real area and stability of microelectrodes; 4) to develop new materials which can operate at high stimulation charge densities for microstimulation; and 5) to provide electrochemical and analytical support for other research activities in the Neural Prosthesis Program at NINDS.

Continuing with work initiated in the previous contract, electrochemical studies of silicon-based probes focussed on long term stability of activated and unactivated Ir sites. Two of four probes had sufficient stability beyond 24 hrs. for extended studies of effect of storage on activation charge capacity and impedance. One of these was studied for 30 days. Two sites on one probe were activated while the other three were left unactivated. Soaking the probe in EBSS for 3 weeks resulted in some decrease in charge capacity of activated sites but very little change in impedance at 1 kHz during this interval when it was measured with an applied bias of 500 mV vs. Ag/AgCl. Sites which had not been activated had CV characteristics of slightly activated Ir, together with an AIROF-like dependence of impedance on potential. Impedances measured with an applied bias of -200 mV vs Ag/AgCl were higher than those measured at 500 mV and tended to increase during 3 weeks of soaking in EBSS.

Although only the impedance data at 1 kHz are shown in this report, measurements were made over the frequency range 0.1 Hz to 100 kHz. During the next quarter impedance data will be analyzed with an equivalent circuit modelling program to try to fit the data to an appropriate equivalent circuit, and determine which element(s) are sensitive to electrolyte composition, long term soaking, potential, etc. Probes will be examined by light and electron microscopy to look for changes in film morphology.

2.0 Ir MICROELECTRODE STUDIES

One task of our new contract is to develop protocols suitable for *in vivo* use for determining the electrochemical real surface areas of Ir microelectrodes and their long term stability. In the previous contract (No. N01-NS-1-2300, Quarterly Report No. 12), we demonstrated that some of the instability problems encountered with multi-site probes from Univ. of Michigan might have been due to inadequate encapsulation of the probe stalk and contact pads, and not due to problems with the Ir sites, *per se*. Covering the stalk and contact pad areas with a layer of dental wax resulted in Ir sites having reproducible responses to $[Ru(NH_3)_6]^{+3}$ for several days making it feasible to carry out long term tests of stability. During this quarter, we continued working with probes supplied to us by Jamie Hetke at the University of Michigan. The probes which were tested this quarter were the following

Probes CN8-48 and CN8-50 from sputter run A.
Probes CN8-47 and CN8-49 from sputter run B.

The sputter run identifications are the same as used previously and were assigned arbitrarily at EIC to distinguish between probes prepared in different sputter runs at Univ. of Michigan. Sites on these probes had geometric areas of 800 μm^2.

Figure 2.1 is a diagrammatic representation of the probe and stalk, with sites identified according to our numbering scheme. The stalks and contact pad area of the probes tested were encapsulated with dental wax as was described previously (NINDS Contract No. NO1-NS-1-2300, Quarterly Report No. 12). Cyclic voltammetry experiments were carried out with an Amel Model 551 potentiostat with potential control by a PAR EG&G Model 175 Universal Programmer and data acquisition with a BT Model 4120 Electronic Recorder, or with a PAR EG&G Model 273 potentiostat controlled with an IBM-compatible computer using PAR Headstart Electrochemistry Software. Impedance spectroscopy was carried out over a frequency range from 0.1 Hz to 100 kHz, using the PAR EG&G Model 273 Potentiostat, Model 5210 Frequency Analyzer and Model 388 Impedance Software. The ac perturbation had an amplitude of 5 mV. All impedance measurements were carried out with the working electrode under potential control. Electrode potential was set either to the open circuit potential as determined prior to the impedance measurement, or to some other potential to establish a specific oxidation state of the Ir oxide. Potentials were referenced to the Ag/AgCl/3M NaCl reference electrode.
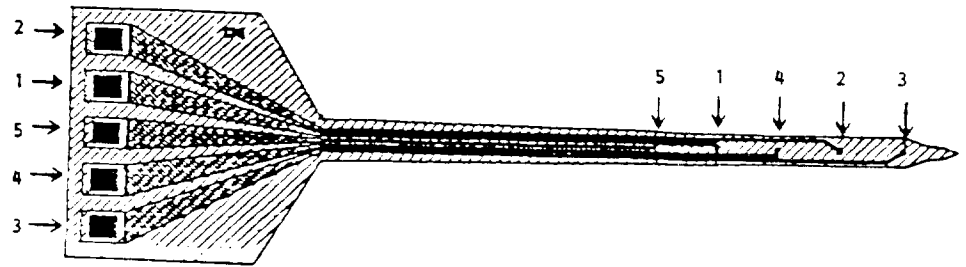
Figure 2.1   Diagrammatic representations of U. Mich. probes
             identifying location of sites tested.

Table 2.1. Summary of electrochemical data for U. Michigan probe CN8-48.

Time	Refer to:	Experimental Protocols	Observation
Day 1	Fig. 2.2	Coat stalk with dental wax. CVs in [Ru(NH₃)₆]³⁺ at 50 mV/s.	All sites: typical, sigmoidal CVs.
Day 2	Fig. 2.2 Fig. 2.3 Table 2.2	CVs in [Ru(NH₃)₆]³⁺ and in PBS solutions. Impedance spectroscopy in PBS on sites 1, 2 and 3 only. Soak overnight in PBS.	All sites: typical, sigmoidal CVs except for site 4. All sites had higher background current in PBS than in [Ru(NH₃)₆]³⁺. Site 4: higher than normal non-faradaic current in [Ru(NH₃)₆]³⁺ suggesting presence of some oxide.
Day 3	Fig. 2.2 Fig. 2.3	After overnight soak, take CVs in PBS and [Ru(NH₃)₆]³⁺ solution.	Sites 1,2,3,5: normal, sigmoidal CVs. Site 4: CVs suggests presence of some oxide. All sites: background current in PBS equals background current in [Ru(NH₃)₆]³⁺.
Day 3 cont'd	Fig. 2.4	Preactivation CVs in 0.3M Na₂HPO₄ at 50 mV/s.	Sites 2,3,5: typical preactivation CVs. Sites 1,4: CVs indicate presence of some oxide.
Day 3 cont'd		Site 2 activated by potential ramp, 100 mV/s, in Na₂HPO₄; site 3 activated by potential pulse.	Sites 2,3: normal activation as indicated by increase in oxide current peaks and overall charge capacity.
Day 4	Fig. 2.4	Post-activation CVs in Na₂HPO₄.	Sites 2,3: typical AIROF CV. Sites 1,4: CVs indicate slight activation. Site 5: typical unactivated Ir.
Day 4 cont'd	Table 2.2	Impedance spectroscopy in Na₂HPO₄.	See Table 2.2. Sites 2 and 3 have lowest impedance consistent with activation. Site 5 has highest impedance, consistent with unactivated Ir. Sites 1 and 4 have impedance intermediate between other sites, consistent with slight activation seen in CVs.
Day 4 cont'd		Post-impedance CVs in PBS at 50 mV/s	Sites 2,3: typical AIROF, no change from previous. Sites 1,4: CVs indicate slight activation. Site 5: CV now indicates slight activation.

7

Table 2.1 Continued.

Time	Refer to:	Experimental Protocols	Observation
Day 4 cont'd	Table 2.2	Impedance spectroscopy in PBS	Ranking of sites by impedance is the same as it was for impedance measurements in Na_3HPO_4. However, all values are slightly lower than those measured in Na_3HPO_4.
Day 7	Fig. 2.5 Fig. 2.6	Store in PBS 2 days. CVs in PBS.	Sites 2,3: typical AIROF CVs. Sites 1,4,5: CVs indicate presence of oxide with site 4 > 1 > 5.
	Table 2.2	Impedance in PBS @ +500 mV and -200 mV vs Ag/AgCl (measurements run overnight).	See Table 2.2. For impedance measured at +500 mV bias: sites 4 and 5 are lower than on Day 4, sites 1 and 2 about the same, and site 1 is higher. All impedances measured at -200 mV bias are higher than those measured at +500 mV.
Day 8		CVs in PBS next A.M.	Indication of Ag contaminant in solution. Sites 2,3: still appear well activated. Sites 1,4,5: still appear slightly activated.
Day 9		Soak 1 day in PBS with numerous solution changes to remove Ag contaminant. CVs taken in fresh PBS.	CVs are clear of any Ag signal. Sites 2,3: remain well activated. Sites 1,4,5: remain slightly activated.
Day 11	Fig. 2.5 Fig. 2.6 Table 2.2	Store in PBS 2 days. Transfer probe to EBSS for CVs and impedance measurements. Impedance measurements in EBSS at +500 mV and -200 mV vs ref.	Sites 2,3: still appear activated but EBSS electrolyte causes a shift in oxide peak potentials such the ΔE_p increases. Sites 1,4: still appear slightly activated with 4 > 1. Site 5: oxide waves are diminished and CV appears more like unactivated Ir. All impedances are higher than those measured in PBS (day 7) with the same applied potential. Impedance of activated sites 2 and 3 are much higher when measured at -200 mV than at +500 mV. This is also true for site 4 which appears activated.
Day 15		Hold electrodes at -300 mV vs. ref. overnight.	

8

Table 2.1 Continued.

Time	Refer to:	Experimental Protocols	Observation
Day 16	Fig. 2.5 Fig. 2.6 Table 2.2	CVs in EBSS on sites 1, 2 and 3 only. Impedance at -300 mV vs. ref. after overnight at -300 mV. After impedance, hold electrodes at +500 mV vs. ref. overnight.	Sites 2, 3: CVs indicate lower oxide currents than previously. Site 1: oxide peaks are diminished and CV appears more like unactivated Ir. All impedances at -300 mV are much higher than day 11 values at -200 mV. Uncertain whether this is due to the longer soak time or the longer time at the low potential.
Day 17	Table 2.2	Impedance at +500 mV vs. ref. after overnight at +500 mV.	All impedances at +500 mV are lower than those at -300 mV. They are only slightly different from day 11 values at +500 mV.
Day 29	Table 2.2	Impedance at +500 mV vs. ref.	All impedances are slightly lower than day 17 values at +500 mV.
Day 29 cont'd	Table 2.2	Impedance at -200 mV vs. ref.	All impedances at -200 mV are much higher than those at +500 mV. They are also higher than day 11 impedances at -200 mV.
Day 30	Fig. 2.5 Fig. 2.6	CVs in EBSS on sites 2, 3, 4, and 5.	Activated sites 2 and 3 show same charge capacity as at 16 days. Unactivated site 4 shows decrease in oxide peaks, but very skewed CV. Unactivated site 5 appears unactivated but with skewed CV.

Table 2.2 Impedance (kΩ) at 1 kHz of Ir sites on Probe CN8-48.
Values in italics are for activated sites.

Time	Conditions during Impedance Measurement	Site 1	Site 2	Site 3	Site 4	Site 5
Day 2	Initial impedance in PBS (at OCP, 45 to 77 mV vs. Ag/AgCl)	101.60	134.10	134.42		180.60
Day 4	In Na_2HPO_4, after activation of sites 2 & 3 (at OCP, -21 to 148 mV vs. Ag/AgCl)	101.63	21.44	28.83	81.90	152.70
Day 4	Measured at +100 mV vs. Ag/AgCl in PBS after activation in Na_3HPO_4	93.62	19.15	24.30	67.53	135.64
Day 7	Measured at +500 mV vs. Ag/AgCl in PBS	104.06	19.75	29.42	59.00	187.43
Day 7	Measured at -200 mV vs. Ag/AgCl in PBS	169.34	57.71	51.76	133.32	243.04
Day 11	Measured at +500 mV vs. Ag/AgCl in EBSS	217.11	47.92	74.92	145.40	243.04
Day 11	Measured at -200 mV vs. Ag/AgCl in EBSS	249.90	113.66	126.09	240.44	295.71
Day 16	Measured at -300 mV vs. Ag/AgCl in EBSS after overnight bias at -300 mV	521.26	399.81	322.50	373.32	NA
Day 17	Measured at +500 mV vs. Ag/AgCl in EBSS after overnight bias at +500 mV	195.93	72.42	103.07	148.13	268.40
Day 29	Measured at +500 mV vs. Ag/AgCl in EBSS	171.45	59.63	93.19	144.76	274.08
Day 29	Measured at -200 mV vs. Ag/AgCl in EBSS	370.99	160.38	190.91	450.64	425.17

Figure 2.2 illustrates the faradaic response to $[Ru(NH_3)_6]^{+3}$ over 3 days of testing. The CVs had the typical sigmoidal shape on all three days, but there were slight and subtle differences in background currents on the different days. A consistent observation was the variation in background current in PBS on days 2 and 3, and an inequality of the cathodic background current in the potential range between +200 mV and 0 mV in PBS and in $[Ru(NH_3)_6]^{+3}$. This inequality is illustrated for site 2 in Figure 2.3. Normally, the background current in PBS will be equal to the non-faradaic current in $[Ru(NH_3)_6]^{+3}$ as is shown in the pair of CVs acquired on day 3. However, in the CVs obtained on day 2, there was a significant inequality observed with all five sites.

Figure 2.4 illustrates the cyclic voltammetric behavior of the five sites in Na_2HPO_4 before and after activation. The day 3 CVs of sites 1 and 4 have some symmetry of small anodic and cathodic current peaks suggesting that these sites are slightly "activated". Note that this is prior to the application of any electrochemical activation protocols. Sites 2, 3 and 5 have similar CV shapes with varying amounts of cathodic current at potentials negative of 0 V. Shown in the same figure are CVs acquired after the activation of sites 2 and 3. At this time, the current in the CV of site 1 has less symmetry, and the CV of site 5 has a more pronounced cathodic current in the potential region below 0 V. The CV of site 4 appears the same as it did prior to activation of sites 2 and 3.

Figure 2.5 illustrates the cyclic voltammetric behavior of the activated sites (sites 2 and 3) in PBS on day 7 and in EBSS on days 11-30. The CVs in PBS are similar to those seen on day 4 in Na_2HPO_4, with the expected shift in peak potentials due to change in pH. The CVs in EBSS on day 11 show the separation of redox peaks typically seen in this electrolyte, but the current magnitude of the major peak is nearly the same as shown in PBS. On days 16 and 30, however, there is a decrease in magnitude of oxide current peaks and overall charge capacity within the voltammetric curve and a slight diagonal orientation of the CVs about the current axis. This type of decrease in charge capacity was observed previously with large electrodes of activated sputtered Ir or activated Ir foil after long term soaking in EBSS. In the case of those large electrodes, the original charge capacity was restored by a single potential cycle between O_2 and H_2 potentials. This restorative treatment was not tried with this probe.

Figure 2.6 illustrates the CVs of sites 1, 4 and 5 in PBS on day 7 and in EBSS on days 11-30. Although these sites were not subjected to an activation protocol, nevertheless the day 7 CVs in PBS all have the appearance of slightly activated Ir, with the CV for site 4 indicating the greatest degree of activation among the 3 sites. The CVs of these sites in EBSS on day 11 are similar to those in PBS, but subsequent CVs show a decrease in charge capacity and a slight diagonal orientation of the CVs about the current axis, similar to what was observed for the activated sites.

The change in CV shape from symmetrical about the current axis to diagonal orientation has previously been attributed to a penetration of electrolyte at the metal/insulator junction. However, the CV changes observed for this probe were not as drastic as those observed for previously tested probes and did not include the large increase in current which accompanied the change in CV shape of earlier probes. In contrast, if one compares the CVs of the unactivated sites by examining only the initial portion of each sweep from 0.1 V to -0.4V and back to 0.2V, the voltammetric curves are similar in current magnitude to the initial curves taken in PBS on day 2 of testing. The major difference between the initial and final traces is in the cathodic tilt of the curves on day 30. This cathodic tilt may be attributed to the presence of O_2, whereas the initial PBS solution was deaerated. The similarity of the overall current scale for the unactivated sites suggests that there has not been a major change in electrochemical surface area of the sites. One way to confirm this would be to repeat the $[Ru(NH_3)_6]^{3+}$ test protocol at the end of the soaking period, and this may be done in future test sequences.

Referring to the impedance data shown in Table 2.2, an examination of the data in each row shows the difference in impedance between sites under the same conditions of measurement. The initial impedance measurements which were made only on sites 1, 2 and 3, were similar in magnitude. After sites 2 and 3 were activated, these consistently had lower impedances than the unactivated sites, regardless of the potential at which the impedance was measured. Site 4 had a lower impedance than the other unactivated sites, which was consistent with the intermediate degree of "activation" shown in its CV behavior.

Figure 2.2 is a graphical comparison of the impedance measurements at +500 mV and -200 mV. Impedances measured with sites biased at +500 mV were lower than those measured with sites biased at -200 mV. The lower impedance at +500 mV probably reflects the greater conductivity of the oxide layer at a potential where it is fully oxidized. However, even sites 1, 4 and 5, which were not intentionally activated, showed a potential dependence in their impedance values supporting the CV data indicating the presence of oxide. Of the three "unactivated" sites, site 4 appeared more characteristic of activated Ir in both its CV behavior and its impedance data. The question arises: How and when was the oxide layer produced on the "unactivated" sites?

Figure 2.7 also shows that the impedances measured in EBSS at +500 mV changed very little between day 11 and day 29, while the impedances measured at -200 mV increased significantly between the same periods, particularly for the unactivated sites. The data shown in the graph were obtained with the sites biased only for a 5 min. equilibration period plus the time for the actual measurement, or about 10 min. total. However, the impedance measurement made on day 16

following an overnight bias at +500 mV was equivalent to that obtained with a short bias period. This result suggests that a brief potential bias to +500 mV vs. Ag/AgCl might be sufficient to restore a site to a low impedance condition.

Light microscopic evaluation of the probe is awaiting the arrival of a new video microscope camera. These will be presented in the next report. Scanning electronmicroscopy will be performed once we are certain that no additional tests are to be done on this probe. The probe had a clump of contaminating "scum" (microbial growth?) encapsulating its tip by the end of the 30 day soak period. We do not know when this material began to accumulate. Unfortunately we could not examine the contaminant closely because it became dislodged from the probe as the probe was removed from solution. It seems likely that the material was loosely attached since it fell off so readily and the changes in voltammograms and impedance, particularly with the +500 mV bias, were relatively small.
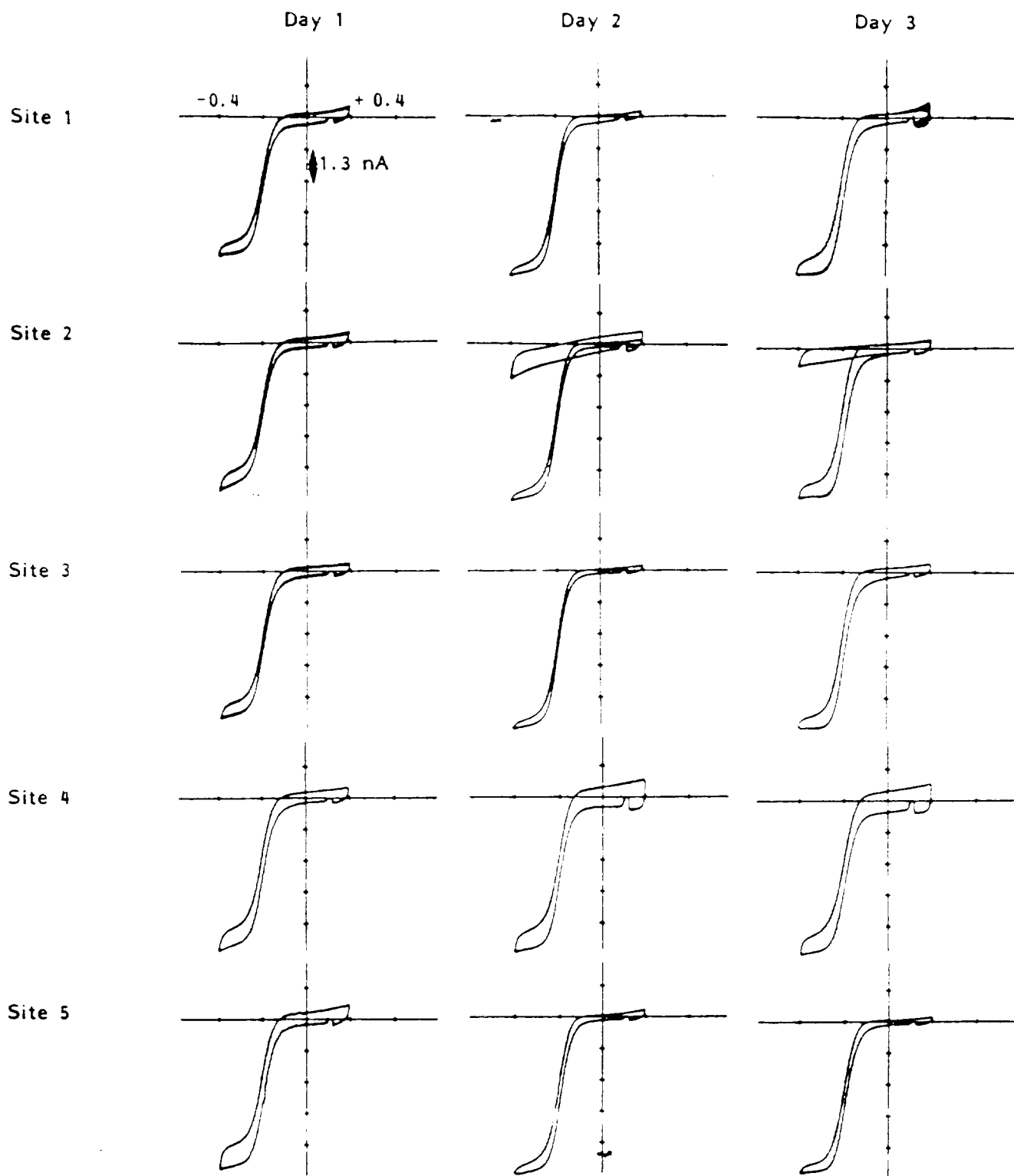
Day 1   Day 2   Day 3

Site 1   −0.4   + 0.4

1.3 nA

Site 2

Site 3

Site 4

Site 5

Figure 2.2.   Cyclic voltammograms acquired at sites on U. Mich. probe CN8-48 in the solution of 1.33 mM $[Ru(NH_3)_6]^{+3}$ in PBS. Background CVs acquired in PBS are also shown for site 2 on days 2 and 3. See also Fig. 2.3. CVs on day 3 were acquired after impedance measurements were made. Sweep rate = 50 mV/s. Potentials referenced to Ag/AgCl/3M NaCl electrode are shown for site 1, day 1 only.

14

1.3 nA

Day 2

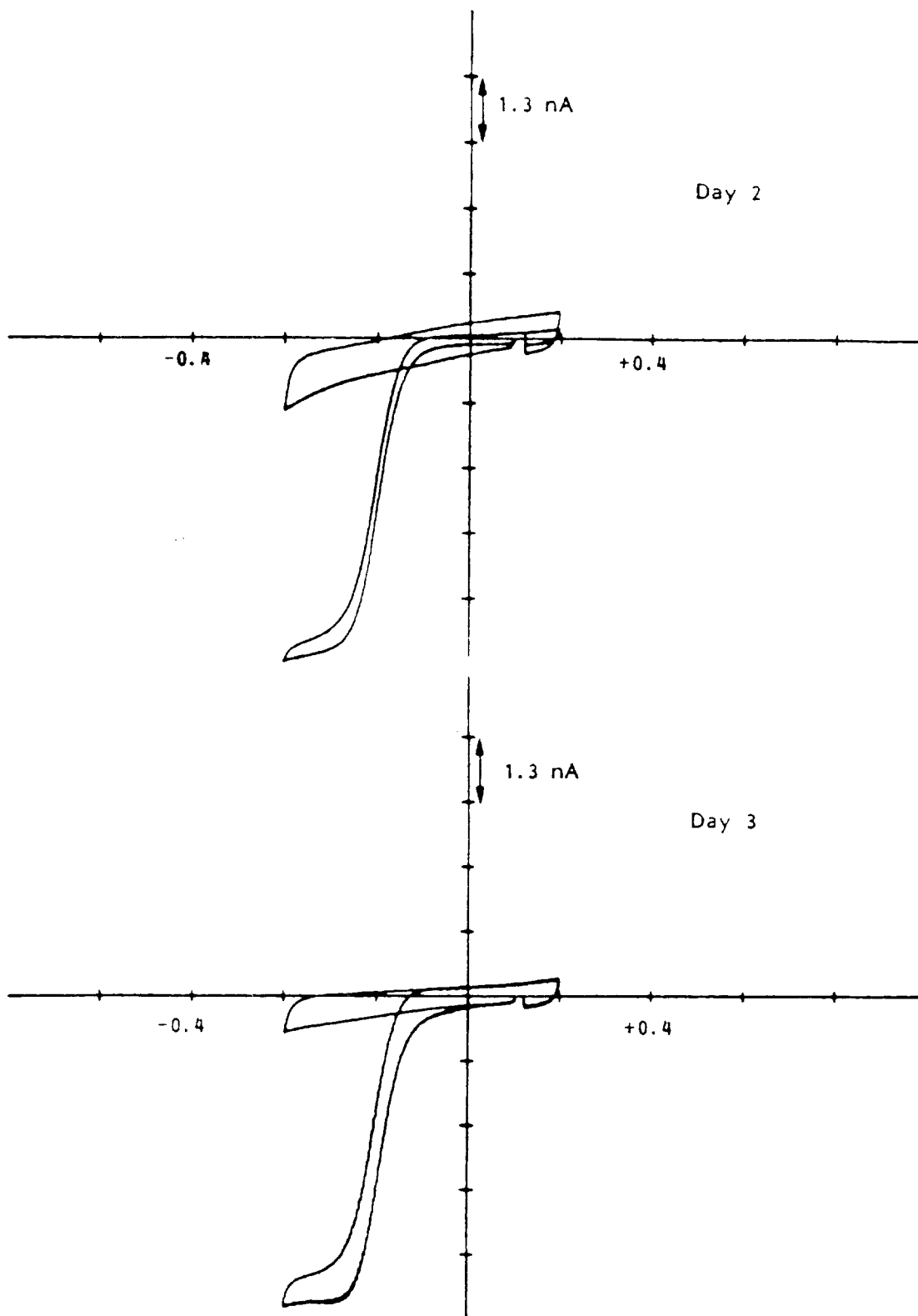−0.4   +0.4

1.3 nA

Day 3

−0.4   +0.4

Figure 2.3.   Cyclic voltammograms acquired at site 2 on U. Mich. probe CN8-48 on days 2 and 3 before and after impedance testing, respectively. Note the change in the background CV due either to the overnight soak or impedance measurement. Same potential window and sweep rate as Fig. 2.2.
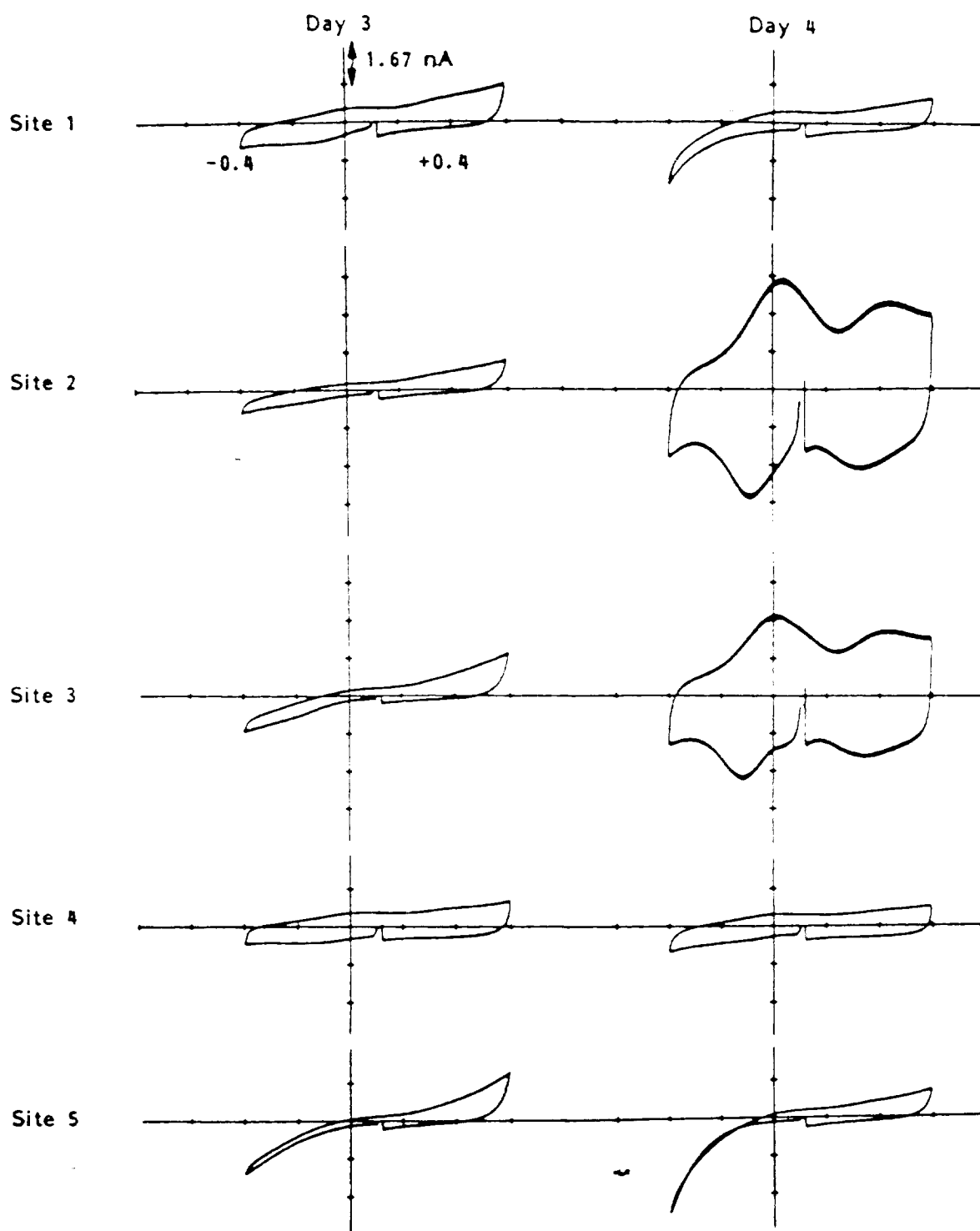
Figure 2.4. Cyclic voltammograms acquired at sites on U. Mich. probe CN8-48 in 0.3 M Na$_2$HPO$_4$ before and after activation. Day 3 CVs were taken before activation. Day 4 CVs were taken after activation of sites 2 and 3, and after overnight soak in 0.3 M Na$_2$HPO$_4$. Potentials referenced to Ag/AgCl/3M NaCl are shown for site 1, day 3 only. Sweep rate = 50 mV/s.
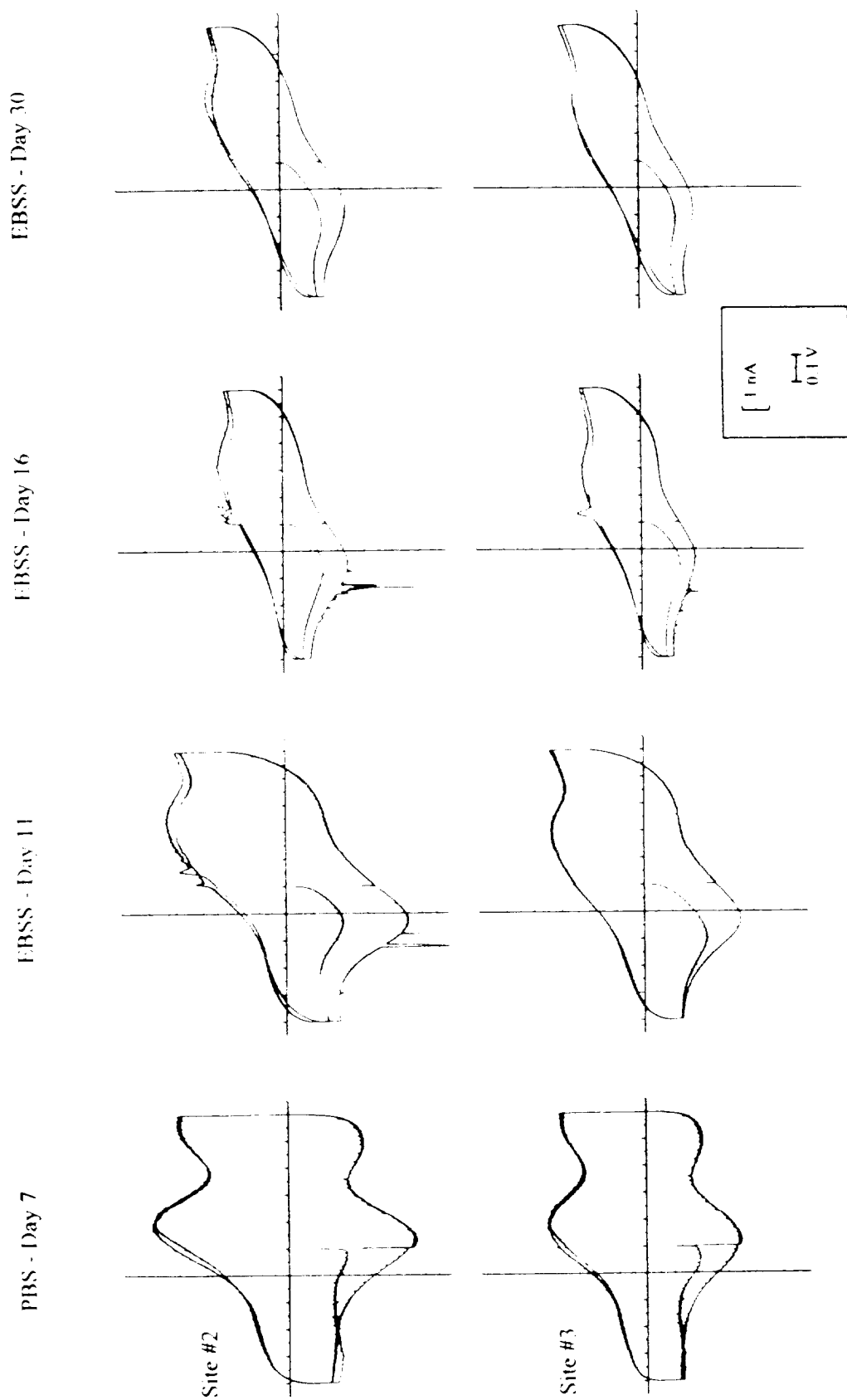
Figure 2.5 Cyclic voltammograms acquired at activated Ir sites on U. Mich. probe CN8.48 at different times during long term soaking. Electrolyte: PBS or EBSS as stated. Sweep rate = 50 mV/s.
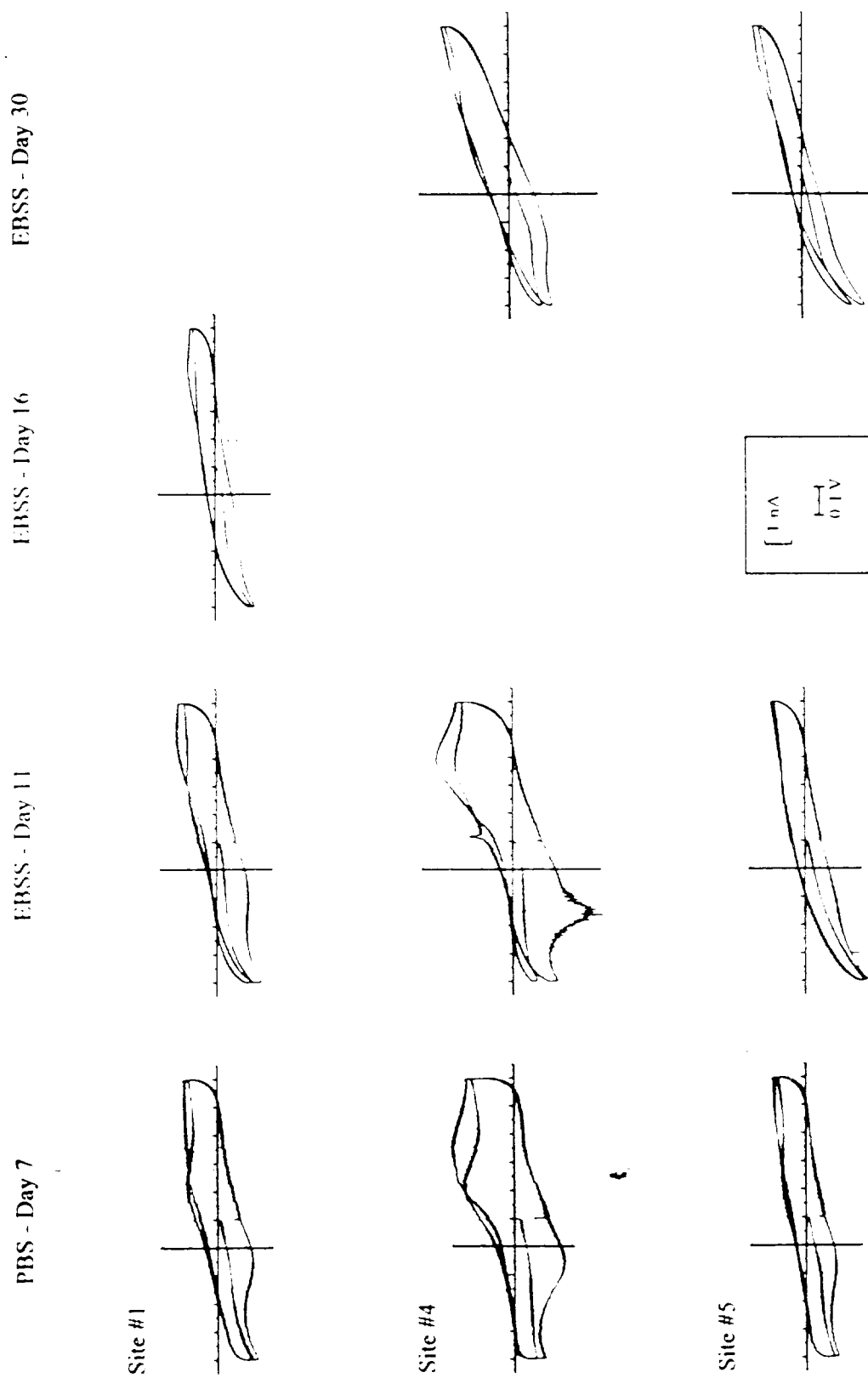
17

Figure 2.6.   Cyclic voltammograms acquired at unactivated Ir sites on U. Mich. probe CN8-48 at different times during long term soaking. Note the small oxide current peaks in CVs of all sites on day 7, even though these sites were not subjected to potential cycles. Electrolyte: PBS or EBSS as stated. Sweep rate = 50 mV/s.
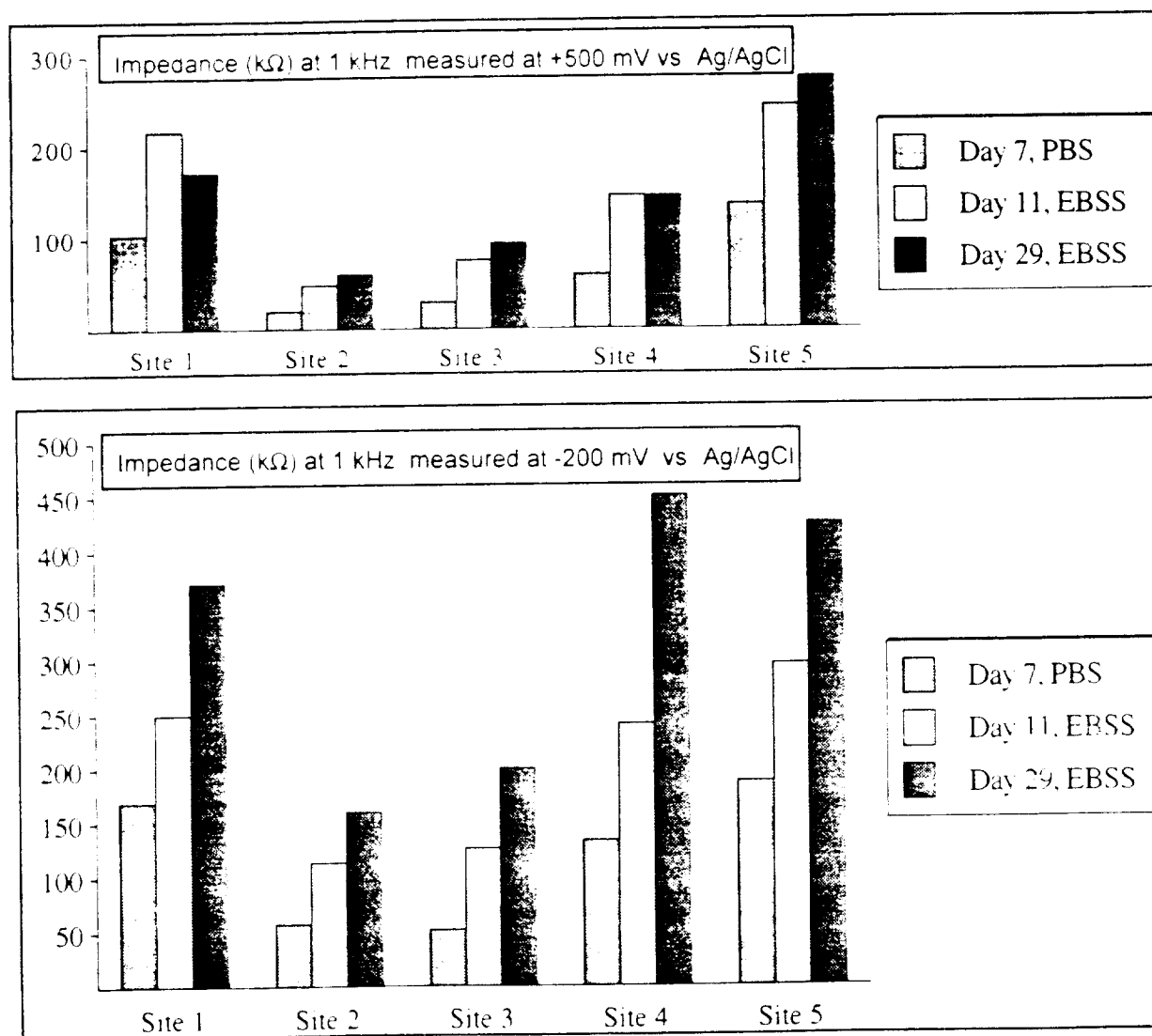
Figure 2.7. Impedance (kΩ) at 1 kHz, measured at +500mV and -200 mV vs Ag/AgCl.

3.0 WORK FOR NEXT QUARTER

Impedance data acquired on probe CN8-48 will be analyzed with an equivalent circuit modelling program to try to fit the data to an appropriate equivalent circuit, and determine which element(s) are sensitive to electrolyte composition, long term soaking, potential, etc. Other probes will be similarly tested to determine how their electrochemical properties and electrochemical surface area are affected by long term soaking.

Two arrays of conically-tipped Ir microelectrodes with Parylene insulation have been submitted by M. Bak, NINDS, for evaluation of their electrochemical real area and integrity of the Parylene/metal junction. Each array contains two microelectrodes. These electrodes will also be tested in the next quarter.